The Effect of Electron Beam Irradiation on the Survival of *Salmonella enterica* Serovar Typhimurium and Psychrotrophic Bacteria on Raw Chicken Breasts Stored at Four Degrees Celsius for Fourteen Days[1]

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ABSTRACT The effect of high-energy electron beam irradiation on the survival of *Salmonella enterica* serovar Typhimurium and psychrotrophic bacteria on commercial chicken breast meat was evaluated. Fresh chicken breast meat was purchased from a local poultry processor, inoculated with 8 $\log_{10}$ cfu/mL *Salmonella*, packaged in Styrofoam trays and over wrapped with a polyvinyl chloride film, and subjected to 0, 1, 2, or 3 kGy of irradiation. The packaged samples were stored at 4°C and analyzed for *Salmonella* Typhimurium and psychrotrophic organisms at 0, 2, 4, 6, 8, 10, 12, and 14 d of storage. Direct plating and enrichment methods were used for *S.* Typhimurium analyses. The direct plating method revealed a 4 log reduction in *Salmonella* for chicken breasts inoculated and treated with 1, 2, or 3 kGy of irradiation. Psychrotrophic counts were conducted at 7°C for 10 d and 25°C for 5 d to determine the effect of incubation methods on the recovery of psychrotrophic organisms. The enrichment method resulted in the repair of injured *Salmonella* cells and an elevated *Salmonella* Typhimurium count for all irradiation dosages when compared with data reported for the direct plating method. In general, psychrotrophic counts increased as storage time increased. However, psychrotrophic counts decreased ($P < 0.05$) as the irradiation dosage increased.

(*Key words*: chicken breast meat, irradiation, psychrotroph, *Salmonella*)

2005 Poultry Science 84:955–958

INTRODUCTION

Contamination of foods, especially those of animal origin, such as meat and poultry, with microorganisms, such as *Salmonella enterica* serovar Typhimurium, pose a public health problem and is a cause of human suffering all over the world (Farkas, 1998). It has been estimated that approximately 800,000 to 4 million cases of *Salmonella* result in 500 deaths in the United States every year (Centers for Disease Control and Prevention, 1999). Therefore, prevention of foodborne illnesses is a major concern of consumers, the meat and poultry processing industry, and regulatory agencies such as the USDA and Food and Drug Administration (Cooke, 1996). Researchers (Abu-Tarboush et al., 1997; Van Calenberg et al., 1999; Lewis et al., 2002) have reported that low doses of irradiation, from 1.0 to 3.0 kGy, are effective in eliminating *Salmonella*, *Yersinia*, *Campylobacter*, and coliforms from poultry meat. However, there is much debate among researchers concerning the most effective dosage of irradiation for specific organisms. Lewis et al. (2002) reported that 60% of inoculated chicken breast samples treated with 1.8 kGy irradiation were negative for *Salmonella*, whereas Abu-Tarboush et al. (1997) reported that a higher dose of irradiation, 2.5 kGy, was needed to eliminate *Salmonella* on chicken.

Another area of concern is the bactericidal effects of irradiation on *Salmonella* vs. the ability to resuscitate injured *Salmonella* cells after irradiation. This study will determine the antimicrobial effects of electron beam irradiation on *Salmonella enterica* serovar Typhimurium and the natural psychrotrophic microflora. Resuscitation of injured *Salmonella* cells after irradiation will also be investigated.

MATERIALS AND METHODS

Storage and Growth of Salmonella Culture

A reference strain of *Salmonella* ATCC 14028[3] was used to prepare the inoculum to test the anti-*Salmonella* effects of 1, 2, and 3 kGy of irradiation on chicken breast fillets.

Received for publication May 28, 2004.
Accepted for publication December 7, 2004.
[1]Florida Agricultural Experiment Station Journal Series R-10266.

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[3]ATCC, Rockville, MD.

Abbreviation Key: HE = Hektoen enteric agar; MIS = Microbial Identification System; PCA = plate count agar; TSB = tryptic soy broth; XLD = xylose lysine desoxycholate.

The MIDI Sherlock Microbial Identification System (MIS)[4] was used to confirm the identity of the isolate. Colonies of the *Salmonella* isolate were inoculated in tryptic soy broth (TSB)[5] and incubated at 37°C for 24 h. After incubation the culture suspension was poured into sterile centrifuge tubes and centrifuged[6] at 5,000 × *g* for 10 min. After centrifuging, the supernatant was discarded, and the pellet was resuspended in 10 mL of sterile distilled water and centrifuged again as previously described. The final supernatant was discarded, and the pellet was resuspended in 1 mL of 3% TSB with 30% glycerol solution in a 2-mL cryovial.[7] Stock cultures were stored at –45°C until ready for use.

A sterile bacteriological loop was used to transfer thawed stock cultures to 3 test tubes containing 10 mL of 3% TSB. The tubes were incubated at 37°C for 24 h. After incubation, serial dilutions of the culture were prepared in 0.1% peptone water[5] and plated on xylose lysine desoxycholate agar (XLD)[5] and Hektoen enteric agar (HE).[5] The plates were incubated at 37°C for 24 h, and colony-forming units of *Salmonella* were counted, recorded, and averaged. This procedure was performed in triplicate. Approximately 10^8 cfu/mL of the *Salmonella* isolate grown in TSB were recovered on the XLD and HE after 24 h of incubation at 37°C.

Sample Preparation

Fresh boneless chicken breasts were purchased from a local poultry processing facility and utilized in the study. Before preparation of the samples, styrofoam trays were individually numbered and labeled. The chicken breasts were cut into 3 to 4 cm wide by 12 to 14 cm long strips with a thickness of 2 to 3 cm and a weight of 25 ± 5 g. Strips that were heavier than the weight specifications were trimmed within the measurement and weight parameters previously mentioned. All strips that did not fall within these parameters were discarded. Each chicken strip was inoculated with approximately 1 mL of *S*. Typhimurium at 1.0×10^8 cfu/mL by spraying with a sterilized 32-oz garden spray bottle. One press of the trigger of the spray bottle was equivalent to 1 mL. After setting for 20 min at room temperature to allow for bacterial attachment, inoculated breasts were packaged in D955 Thermoform Laminate shrink film[8] (oxygen transfer rate: 3,000 mL/m^2 per 24 h at 0% relative humidity). The packages were stored in a freezer 0°C overnight (approximately 18 to 24 h).

Irradiation of Chicken Breasts

Frozen inoculated breast strips were placed in a cooler with ice and transported to the Florida Accelerator Services and Technology linear accelerator in Gainesville, FL. The chicken breast samples were loaded onto trays in a single layer, placed on the linear accelerator conveyor belt, and irradiated with accelerated electrons using a Linatron 1,000-A, 5.2-MeV, 1.4-kW linear accelerator.[9] The samples were subjected to 0, 1, 2, or 3 kGy dosages of irradiation. The speed of the belt was set so that each passage took approximately 10 min, and each package received 1 kGy of irradiation on each passage. Upon completing the desired passes, each package was returned to the cooler along with ice and transported to the meat science microbiology laboratory for analysis. Five treatments were prepared and included 1) negative control, no inoculum, no irradiation; 2) inoculum, 0 kGy; 3) inoculum, 1.0 kGy; 4) inoculum, 2.0 kGy; and 5) inoculum, 3.0 kGy. Chicken breast fillets (3 strips/d per treatment) were analyzed after 0, 2, 4, 6, 8, 10, 12, and 14 d storage at 4°C for *Salmonella* Typhimurium and total psychrotrophic organisms. Direct and enrichment methods were used for *S*. Typhimurium analysis. The direct method was used to determine the viable *Salmonella* count after irradiation, and the enrichment method was used to determine the effectiveness of irradiation when the microflora was subjected to favorable nutrient conditions. Selected bacterial isolates that survived the irradiation process were isolated and identified using the API 20E testing system.[10] Identification of selected *S*. Typhimurium isolates was confirmed using the MIDI Sherlock MIS.[4] The MIDI identifies microorganisms based on the unique fatty acid pattern of each strain. The system uses gas chromatographs, microbial databases, and pattern recognition software to identify each strain.

Enrichment for Salmonella

Twenty-five–gram samples of the irradiated chicken breasts were blended with 225 mL of lactose broth and allowed to set at room temperature for 60 min. After incubation, the pH of the broth was determined and adjusted to a desired pH of 6.8 ± 0.2 by adding 1 *N* NaOH. The sample was then incubated at 35°C for 24 h. After a 24-h incubation, 0.1 mL of the enrichment broth was transferred to 10 mL of Rappaport-Vassiliadis broth,[5] which was then incubated at 43°C for 24 h. Serial dilutions were prepared with 0.1% peptone water. Appropriate predetermined dilutions were surface plated onto XLD and HE agar plates and incubated for 24 h at 37°C. After 24 h, colonies were recorded as presumptive positive or negative.

Recovery of S. Typhimurium and Psychrotrophs

Twenty-five grams of chicken were aseptically placed in sterile 18 × 30 cm stomacher bags containing 18 mL of sterile 0.1% peptone water. The bags were gently massaged by hand for 2 min to recover surface bacteria. Serial dilutions were prepared using 0.1% peptone water and plated in duplicate on XLD and HE agar to enumerate *Salmonella* and on plate count agar (PCA)[5] to enumerate psychrotrophs. The XLD and HE plates were incubated at 37°C for 24 h, and PCA plates were incubated at 7°C for 10 d

[4]MIDI, Inc., Newark, DE.
[5]Difco Laboratories, Detroit, MI.
[6]Sorvall RC-5B, Dupont Instruments, Newtown, CT.
[7]Corning Incorporated, Corning, NY.
[8]Cryovac Seal Air Corporation, Simpsonville, SC.
[9]L1000A, Varian, Palo Alto, CA.
[10]bioMerieux SA, Marcey l'Etoile, France.

TABLE 1. Mean *Salmonella entericia* serovar Typhimurium counts (log_{10}) recovered using direct plating methods for chicken breasts inoculated with *S. Typhimurium* treated with 0, 1, 2, or 3 kGy dosages of electron beam irradiation and stored at 4°C for 0 to 14 d

	Storage (d)								
Treatment	0	2	4	6	8	10	12	14	SEM
	(log_{10} cfu/g)								
0 kGy, inoculated	4.4[a]	6.1[a]	5.3[a]	4.7[a]	4.5[a]	3.1[a]	2.6[a]	1.9[a]	0.5
0 kGy, uninoculated	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]	1.0[a]	
1 kGy, inoculated	0.4[b]	2.9[ab]	0[b]	0[b]	0[b]	0[b]	0[b]	0[a]	
2 kGy, inoculated	0[b]	0.2[b]	0.2[b]	1.1[b]	0[b]	0[b]	0[b]	0[a]	
3 kGy, inoculated	0[b]	1.3[b]	0[b]	0[b]	0[b]	0[b]	0[b]	0[a]	

[a,b]Means within the same column without a common superscript letter differ significantly ($P < 0.05$).

or 25°C for 5 d. After the appropriate incubation time, typical *Salmonella* colony-forming units from the XLD and HE plates and psychrotrophic counts (cfu/mL) from PCA plates were recorded.

Data Analysis

A total of 360 samples were analyzed (5 treatments, 8 sampling d, 3 subsamples per treatment, and 3 trials) in this study. The experimental design was a complete randomized block. Statistical analyses were conducted on the *Salmonella* and aerobic psychrotrophic bacteria population data. The PROC GLM program of the SAS software was used to statistically analyze for trial, day, treatment, and treatment-by-day effects. Comparisons of means were performed using the least squares means statement of SAS (1996). Treatment effects and differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Recovery of S. Typhimurium

Direct Plating Method. The uninoculated samples were negative for *S*. Typhimurium growth (Table 1). The irradiation dosages of 1 to 3 kGy did not completely inactivate all *S*. Typhimurium cells. The data revealed significant differences due to trials. This difference could be largely attributed to the lower ($P < 0.05$) *S*. Typhimurium count for trial 2 when compared with trial 1. However, the differences among all trials were less than 1 log. The differences in treatment ($P = 0.0001$) and storage ($P = 0.0008$) were due to the significantly higher *S*. Typhimurium counts reported for the inoculated unirradiated treatment when compared with all other treatments on all storage days. The data demonstrated that except for the treatment with 1 kGy, no *S*. Typhimurium was recovered on d 0. The *S*. Typhimurium counts remained at 1 log or less for the inoculated samples treated with 2.0 and 3.0 kGy of irradiation through 14 d storage. This decrease in *S*. Typhimurium represented a 4 to 5 log reduction for the 2.0 and 3.0 kGy treatments when compared with the inoculated control.

Enrichment Method. In an effort to resuscitate (or revive) *S*. Typhimurium cells that might have been injured during the irradiation process, chicken breast fillet samples from each treatment were enriched prior to plating on selective media. The enrichment method detected more positive samples over the course of the study (Table 2). The data demonstrated that cells thought to be killed were actually injured and could be resuscitated when placed under favorable growth conditions. This observation agrees with the findings of Lamuka et al. (1992) who reported that cells not killed, but injured, during irradiation can be resuscitated when placed under favorable growth conditions. As was true for the direct plating method, no positive *S*. Typhimurium was detected on the uninoculated samples but was detected on all inoculated, and inoculated-irradiated samples. Positive samples were detected for the inoculated control, 1.0 kGy, and 2.0 kGy treatments on all days; positive samples were detected on all days for the 3.0 kGy treatment except for d 8 and 14. Thayer et al. (1992) reported that survival of *S*. Typhimurium on chicken wings decreased significantly as the gamma radiation dose increased when inoculated with 100 cfu/g of *S*. Typhimu-

TABLE 2. Presence (+) or absence (–) of *Salmonella* recovered using enrichment methods for chicken breasts inoculated with *Salmonella* treated with 0, 1, 2, and 3 kGy dosages of electron beam irradiation and stored at 4°C for 14 d

	Storage (d)							
Treatment	0	2	4	6	8	10	12	14
	(log_{10} cfu/g)							
0 kGy, inoculated	+	+	+	+	+	+	+	+
0 kGy, uninoculated	–	–	–	–	–	–	–	–
1 kGy, inoculated	+	+	+	+	+	+	+	+
2 kGy, inoculated	+	+	+	+	+	+	+	+
3 kGy, inoculated	+	+	+	+	–	+	+	–

TABLE 3. Mean psychrotrophic counts (log_{10}) for chicken breast fillets treated with 0, 1, 2, and 3 kGy dosages of electron beam irradiation and stored at 4°C for 14 d, plated onto plate count agar, and incubated at 7°C for 10 d

Treatment	Storage (d)								SEM
	0	2	4	6	8	10	12	14	
	(log_{10} cfu/g)								
0 kGy, inoculated	3.8[a]	6.0[a]	7.7[a]	9.1[a]	8.6[a]	8.9[a]	8.8[a]	9.4[a]	0.5
0 kGy, uninoculated	3.7[a]	5.7[a]	7.5[a]	8.7[b]	9.1[a]	9.4[a]	9.3[a]	9.3[a]	
1 kGy, inoculated	0.5[b]	2.4[b]	3.2[b]	4.3[b]	5.2[b]	6.0[b]	5.9[b]	7.4[ab]	
2 kGy, inoculated	0[b]	0.3[c]	0.8[c]	1.5[c]	3.4[c]	4.0[bc]	3.0[b]	5.1[b]	
3 kGy, inoculated	0[b]	0.1[c]	0.1[c]	0.5[c]	2.0[c]	2.4[cd]	4.0[b]	2.0[c]	

rium with no viable *S*. Typhimurium organisms detected at absorbed gamma radiation doses exceeding 1.42 kGy. Fu et al. (1995) reported that inoculation of pork chops and hams with *S*. Typhimurium and irradiation with 0.75 or 0.90 kGy resulted in 1 and 3 log reductions for chops and hams, respectively. Colonies counted and reported as *S*. Typhimurium from the direct plate methods were confirmed as *S*. Typhimurium using the MIDI MIS rapid detection method.

Recovery of Psychrotrophic Bacteria

Psychrotrophic counts were similar ($P > 0.05$) for PCA plates incubated at 7°C for 10 d and 25°C for 5 d. (Table 3). Therefore, the results and trends discussed are the same for both incubation methods. Colonies isolated from plates stored at both incubation methods were identified as *Pseudomonas fluorescens*, *Hafnia alvei*, *Klebsiella pneumoniae*, *Micrococcus* spp., *Enterobacter cloacae*, *Serratia liquefaciens*, and *Serratia plymuthica*. The significant treatment-by-day interaction ($P = 0.0001$) was attributed to increased psychrotrophic counts as storage time increased and decreased as the dosage level of irradiation increased. No differences ($P > 0.05$) in psychrotrophic counts were revealed among the controls, 0 kGy inoculated and 0 kGy uninoculated. Both treatments had higher ($P < 0.05$) psychrotrophic counts than samples inoculated and irradiated with 1, 2, and 3 kGy. Irradiation of samples with 2 and 3 kGy resulted in lower ($P < 0.05$) psychrotrophic counts when compared with the control samples and samples irradiated with 1.0 kGy. The most effective irradiation treatment was 3.0 kGy. Treatments that received 3 kGy were lower ($P < 0.05$) than all other treatments. The data also demonstrated that after 14 d of storage the psychrotrophic counts for samples irradiated with 2.0 and 3.0 kGy were less than 6 to 7 log10 cfu/g, which is usually necessary for the onset of spoilage in fresh meat. Kampelmacher (1984) reported that a dosage of 2.5 to 5 kGy extended the shelf life of poultry at chill temperatures from 6 to 14 d. Lamuka et al. (1992) determined that treating broiler chicken meat with 2.5 kGy of gamma irradiation resulted in increased shelf life by more than 9 d.

Our study revealed that irradiation dosages of 2.0 and 3.0 kGy were effective in achieving 3 to 5 log reductions in *S*. Typhimurium populations on chicken breast meat fillets. The importance of conducting enrichment studies to determine the bactericidal effects of the irradiation process was also revealed. The study revealed that all doses of irradiation were effective in reducing the growth of psychrotrophic bacteria. However, none of the dosages completely eliminated psychrotrophs. Based on the data revealed in this study, irradiation was an effective tool for reducing *S*. Typhimurium and spoilage organisms, but it did not completely eliminate *S*. Typhimurium or spoilage organisms. Therefore, refrigeration, proper handling, and storage of irradiated foods are essential to ensure a safe product.

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